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Original Article

EGF rs4444903A allele may decrease hepatocellular carcinoma risk in Chinese individuals: a meta-analysis

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Abstract: The epidermal growth factor (EGF) pathway stimulates the proliferation and differentiation of epidermal and epithelial tissues, and plays an important role in tumorigenesis, including the initiation and development of hepatocellular carcinoma (HCC). Since the association between EGF rs4444903A/G polymorphism and the risk of HCC is still controversial and ambiguous, this meta-analysis aimed to evaluate and confirm this relationship. We conducted a literature search in the PubMed and WanFang databases, covering all papers published by July 10, 2014. Overall, 9 case-control studies comprising 1,874 patients and 2,302 healthy controls were retrieved based on the search criteria for HCC susceptibility related to the rs4444903A/G polymorphism. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the strength of this association. We found that in the overall analysis, EGF rs4444903A/G polymorphism decreased HCC risk (A-allele vs. G-allele, OR = 0.93, 95% CI = 0.88-0.98, $P_{heterogeneity}$ = 0.118). However, this finding was not observed in Chinese individuals, who carried the A allele (AA+AG vs. GG, OR = 0.93, 95% CI = 0.88-0.99, $P_{heterogeneity}$ = 0.249). This trend was observed in both hospital-based and population-based subgroups. Our study showed that the A allele of EGF rs4444903 was a poor protective factor of HCC risk in Chinese individuals.

Keywords: Chinese, epidermal growth factor, hepatocellular carcinoma, meta-analysis, polymorphism, risk

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and accounts for more than 600,000 deaths annually, more than half of which occur in China [1]. Most patients with HCC have chronic liver disease, especially liver cirrhosis, which is mainly attributed to hepatitis virus infection [2]. In the US and Europe, chronic hepatitis C virus (HCV) infection represents the main risk factor [3], while in Asia and Africa, chronic hepatitis B virus (HBV) infection is the leading risk factor [4]. Only a few patients with HCC are candidates for potentially curative treatments of resection, transplantation, and ablation. Because of its poor prognosis, HCC is the third leading cause of cancer-related deaths worldwide [5]. Therefore, identification of biomarkers related to an increased risk of HCC would better define the population at the highest risk for HCC and may enable the selection of suitable strategies for prevention and treatment of HCC. It is becoming increasingly apparent that the heritability of the majority of population-attributable cancers (such as HCC) is related not to the rare deleterious gene defects but to polymorphic variations in the DNA sequence [6].

Recently, several studies have investigated the risk of HCC associated with the polymorphism of rs4444903A/G in the epidermal growth factor (EGF) gene. EGF, located in chromosome 4q25-q27 [7], contains 24 exons and 23 introns, and encodes a ligand for the EGF receptor (EGFR). As an endocrine growth factor, EGF can activate DNA synthesis, cellular differentiation, and proliferation via binding to EGFR. EGF is overexpressed in malignant glioma, and breast, pancreas, and liver carcinomas, indicat-

ing its vital role in malignant cell transformation, and tumor initiation and development by promoting cell division via autocrine or paracrine pathways, EGFR gene amplification, and by activating mutations [8, 9]. Further, EGF is a mitogen for the cultured adult and fetal hepatocytes, and its expression level can be upregulated during liver regeneration [10, 11]. It has been reported that transgenic mice with liver-targeted overexpression of the secreted EGF fusion protein develop HCC [12]. Gene expression profiles comparing normal liver tissue with liver tumors in these mice suggest the role of an autocrine mechanism during EGF-induced hepatocarcinogenesis [13].

Shahbazi et al. first reported a functional single nucleotide polymorphism (SNP) involving an A to G mutation at position 61 in the 5'-untranslated region of the EGF gene (rs4444903) [14]. They demonstrated that the G allele showed an increased EGF protein expression by affecting DNA folding or mRNA transcription *in vitro*, and revealed that patients with malignant melanoma of the skin had a significantly higher frequency of G allele compared with the general population. Another recent study showed a relationship between this functional polymorphism and the risk for development of HCC [15]. In this study consisting of 207 patients, it was observed that the frequencies of GG or (GG+GA) genotypes were associated with the risk for aggressiveness of HCC in liver cirrhosis, through modulation of EGF levels.

Two meta-analyses proved that rs4444903G allele was a risk factor for HCC, while rs4444903A allele was a protective factor [16, 17]. However, in these studies, the control group was not completely devoid of patients with hepatitis or liver cirrhosis, which may have increased the publication bias and heterogeneity. Thereafter, several novel studies were published; it is necessary to collate the data from all previous related studies to conduct an updated analysis, while considering the influence of the controls. To the best of our knowledge, thus far, a total of 9 case-control studies on HCC have been reported.

Materials and methods

Identification and eligibility of relevant studies

We conducted literature searches in the PubMed and Wan Fang databases (last search updated on July 10, 2014), using keywords such as 'EGF' or 'epidermal growth factor', 'polymorphism' or 'variant' and 'hepatocellular carcinoma' or 'liver disease', without imposing any restriction on the language or year of publication. Using these terms, a total of 15 articles were retrieved, 7 of which adhered to the inclusion criteria of this study. We also screened the references of the retrieved articles and reviewed them manually.

Inclusion and exclusion criteria

Studies that assessed the correlation between HCC and EGF rs4444903A/G polymorphism, case-control studies, studies that had sufficient genotype numbers for patients and controls, and those in which the genotype distributions of controls were consistent with the Hardy-Weinberg equilibrium (HWE) were included in our analysis. Studies that had no control population, those with no available genotype frequency, and duplicate publications were excluded from our analysis.

Data extraction

Two investigators independently extracted all data, ensuring compliance with the selection criteria. The following items were collected: first author's last name, year of publication, country of origin, ethnicity, total no. of patients/controls, source of controls, HWE of controls, and genotyping method.

Statistical analysis

Odds ratio (OR) and 95% confidence interval (CI) were used to measure the strength of the association between EGF rs4444903A/G polymorphism and HCC based on the genotype frequencies in patients and controls. Initially, subgroup analysis stratified by ethnicity was performed. According to the source of controls, the articles were defined as population-based (PB) or hospital-based (HB).

The statistical significance of the summary OR was determined by the *Z*-test. Heterogeneity assumption was evaluated with a chi-square-based *Q*-test. $P > 0.05$ for the *Q*-test indicated a lack of heterogeneity among the studies. In order to better evaluate the extent of heterogeneity among the studies, the I^2-test was used. As a guide, I^2 values < 25% were considered

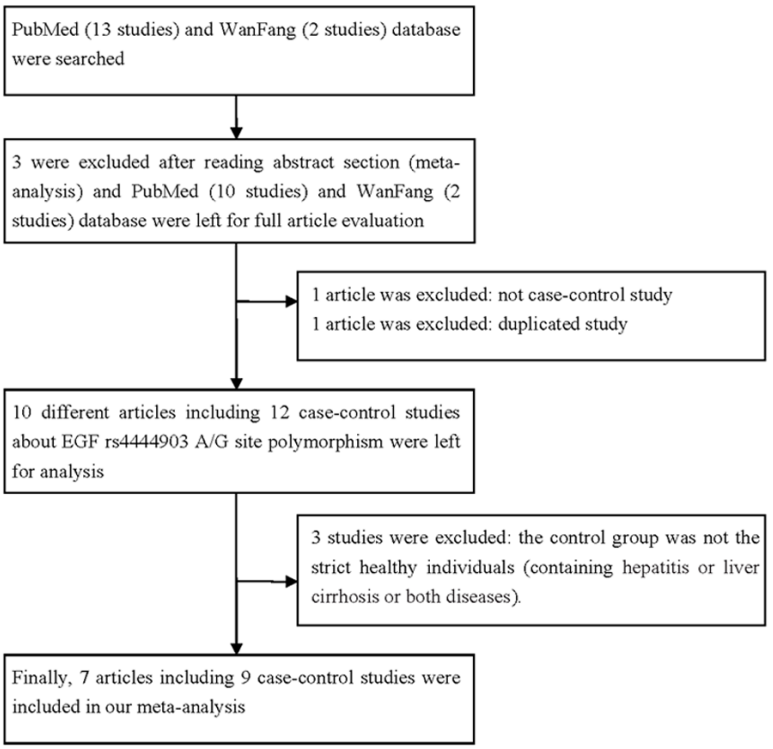

Figure 1. Flowchart illustrating the search strategy for EGF rs4444903A/G polymorphism and the risk of HCC.

Table 1. Characteristics of studies of EGF rs4444903A/G polymorphism included in this meta-analysis

First author/ Year	Country/Ethnicity	Case/ Control	Source of Control	Genotyping Method	HWE of Control
Abbas/2012	Egypt/Caucasian	20/20	HB	PCR-RFLP	0.371
Wu/2013	China/Chinese	404/623	HB	TaqMan	0.094
Qi/2009	China/Chinese	215/208	HB	PCR-RFLP	0.615
Li/2009	China/Chinese	186/186	HB	PCR-RFLP	0.564
Chen/2011	China/Chinese	120/120	HB	PCR-RFLP	0.971
Wang/2009	China/Chinese	376/477	PB	PCR-RFLP	0.335
Wang/2009	China/Chinese	186/198	PB	PCR-RFLP	0.550
Yuan/2013	USA/Chinese	250/245	HB	TaqMan	0.533
Yuan/2013	USA/Mixed	117/225	PB	TaqMan	0.162

Population-based (PB); hospital-based (HB); polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

'low', those ~50% were considered 'moderate' and those > 75% were considered 'high' [18]. If P was ≤ 0.05 or I^2 was ≥ 50%, a random-effects model using the DerSimonian and Laird method [19], which yields wider confidence intervals, was adopted. Alternatively, if P was > 0.05 or I^2 was < 50%, a fixed-effects model using the Mantel-Haenszel method [20] was used.

For EGF rs4444903A/G, we investigated the relationship between genetic variants and HCC risk in allelic contrast (A-allele vs. G-allele), homozygote comparison (AA vs. GG), heterozygote comparison (AG vs. GG), dominant genetic model (AA+AG vs. GG), and recessive genetic model (AA vs. AG+GG). In addition, Begg's funnel plots and Egger's regression test were used to assess the publication bias. $P < 0.05$ in both tests indicated the presence of publication bias [21, 22].

Significant departures of allele frequencies of EGF polymorphism from expectation under HWE were assessed in controls using the Pearson's chi-square test. $P < 0.05$ was considered statistically significant. All statistical tests were performed using Stata software (version 10.0; StataCorp LP, College Station, TX, USA).

Genotyping method

Genotyping for rs4444903-A/G SNP of EGF gene was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the TaqMan method.

Results

Study selection and characteristics of the meta-analysis

A total of 15 published studies assessing the association of EGF rs4444903A/G polymorphism and HCC were retrieved by searching the PubMed and Wan Fang databases. Through abstract appraisal, 12 articles were identified as eligible for full-text appraisal. Of these, 5 articles (1 duplication, 1 case only study, and 3 without strictly healthy controls [15, 23, 24])

Table 2. Stratified analyses of the EGF rs4444903A/G polymorphism on HCC risk

Genetic model (No. of studies:Cases/Controls)	Main effects of EGF rs4444903A/G polymorphism in HCC				
	OR (95% CI)	$P_{heterogeneity}$	*P*	I^2	Analysis model
Total (9:1874/2302)					
Allelic contrast	0.93 (0.88-0.98)	0.118	0.010	37.7	F
Homozygote comparison	0.87 (0.77-0.97)	0.145	0.013	34.1	F
Heterozygote comparison	0.94 (0.88-1.01)	0.231	0.091	23.9	F
Dominant genetic model	0.94 (0.90-0.99)	0.138	0.023	37.7	F
Recessive genetic model	0.89 (0.78-1.01)	0.238	0.079	23.1	F
Ethnicity					
Chinese (7:1737/2057)					
Allelic contrast	0.93 (0.88-0.99)	0.429	0.015	0.0	F
Homozygote comparison	0.86 (0.76-0.97)	0.204	0.017	29.4	F
Heterozygote comparison	0.93 (0.87-1.00)	0.050	0.015	14.1	R
Dominant genetic model	0.93 (0.88-0.99)	0.249	0.016	23.6	F
Recessive genetic model	0.91 (0.79-1.05)	0.449	0.196	0.0	F
Source of control					
Hospital-based (6:1195/1402)					
Allelic contrast	0.95 (0.89-1.01)	0.124	0.118	42.2	F
Homozygote comparison	0.87 (0.76-0.98)	0.065	0.026	51.9	R
Heterozygote comparison	0.98 (0.90-1.06)	0.604	0.601	0.0	F
Dominant genetic model	0.97 (0.91-1.03)	0.495	0.268	0.0	F
Recessive genetic model	0.74 (0.51-1.07)	0.083	0.110	48.6	R
Population-based (3:679/900)					
Allelic contrast	0.90 (0.81-0.99)	0.209	0.031	36.2	F
Homozygote comparison	0.86 (0.68-1.10)	0.446	0.228	0.0	F
Heterozygote comparison	0.83 (0.58-1.19)	0.099	0.309	56.7	R
Dominant genetic model	0.90 (0.83-0.99)	0.031	0.025	71.2	R
Recessive genetic model	0.87 (0.67-1.14)	0.980	0.313	0.0	F

were excluded. Finally, 7 articles involving 9 case-control studies were finalized, and data from these were extracted for further assessment in our meta-analysis (**Figure 1**). All essential information [25-31] is listed in **Table 1**. If a study consisted of two or more races as its research subjects, we considered it as mixed population. Two articles [25, 28] that consisted of two groups each were considered as two independent case-control studies. The distribution of genotypes in all controls was in agreement with the HWE.

Quantitative data synthesis and test of heterogeneity

Table 2 shows the summary OR of EGF rs4444903A/G based on 1,874 HCC patients and 2,302 healthy controls. We observed a decreased association between the EGF rs4444903A/G polymorphism and HCC in the total population (AA vs. GG, OR = 0.76, 95% CI = 0.61-0.94, $P_{heterogeneity}$ = 0.540, *P* = 0.013, I^2 = 0.0; AA+AG vs. GG, OR = 0.94, 95% CI = 0.90-0.99, $P_{heterogeneity}$ = 0.138, *P* = 0.023, I^2 = 37.7; A-allele vs. G-allele, OR = 0.88, 95% CI = 0.80-0.97, $P_{heterogeneity}$ = 0.204, *P* = 0.010, I^2 = 27.0, **Figure 2**). Given the ethnic differences in the allele frequency of this sequence variant, we evaluated the effect of EGF rs4444903A/G polymorphism in Chinese, European, and Mixed population. The association of rs4444903A/G polymorphism with decreased HCC risk in Chinese population was observed under homozygote comparison (OR = 0.75, 95% CI = 0.60-0.95, $P_{heterogeneity}$ = 0.956, *P* = 0.017, I^2 = 0.0), heterozygote comparison (OR = 0.93, 95% CI = 0.87-1.00, $P_{heterogeneity}$ = 0.050, *P* = 0.015, I^2 = 14.1), dominant genetic model (OR = 0.93, 95% CI = 0.88-0.99, $P_{heterogeneity}$ = 0.249, *P* = 0.016, I^2 = 23.6), and allelic contrast (OR = 0.88, 95% CI = 0.80-0.98, $P_{heterogeneity}$ = 0.788, *P*

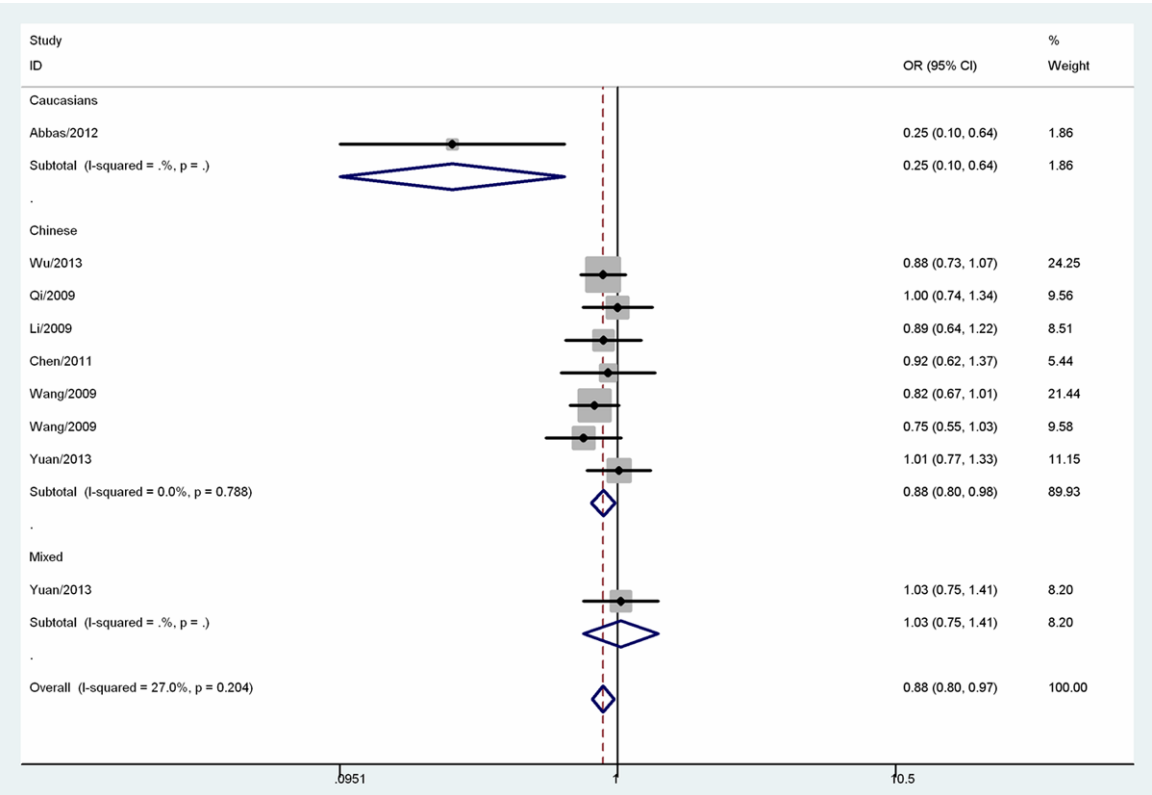

Figure 2. Forest plot of HCC risk associated with the EGF rs4444903A/G polymorphism (G vs. A) by ethnicity subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

= 0.015, I^2 = 0.0, **Figure 2**). Summary OR for rs4444903A/G polymorphism stratified by source of control was evaluated. We also observed decreased association in homozygote comparison (OR = 0.87, 95% CI = 0.76-0.98, $P_{heterogeneity}$ = 0.065, P = 0.026, I^2 = 51.9) in HB studies, while significant relationships in allelic contrast (OR = 0.90, 95% CI = 0.81-0.99, $P_{heterogeneity}$ = 0.209, P = 0.031, I^2 = 36.2) and dominant genetic model (OR = 0.90, 95% CI = 0.83-0.99, $P_{heterogeneity}$ = 0.031, P = 0.025, I^2 = 71.2) were found in PB studies.

Sensitivity analysis and publication bias

No other single study influenced the summary OR qualitatively, as indicated by the sensitivity analysis (**Figure 3**). Begg's funnel plot and Egger's test were performed to assess the publication bias of the literatures. The shape of the funnel plots seemed asymmetrical in allele comparison for EGF rs4444903A/G polymorphism, suggesting no publication bias (e.g., z = -0.83, P = 0.404 for allelic contrast, **Figure 4**). Subsequently, Egger's test was used to provide statistical evidence of funnel plot symmetry. However, no evidence of publication bias was detected (e.g., t = -1.44, P = 0.196 for allelic contrast, **Figure 5**; **Table 3**).

Discussion

HCC is a complex, heterogeneous malignancy, the pathogenesis of which involves multiple genetic and epigenetic alterations, and modulation of molecular signaling pathways implicated in malignant transformation of hepatocytes and tumor progression [32]. Dysregulation of the EGF/EGFR signaling pathway is thought to be important in early hepatocarcinogenesis [33, 34]. EGF can activate multiple signaling pathways involved in cell proliferation, differentiation, and tumorigenesis. Overexpression of EGF is also associated with the growth and invasion of some malignant tumors via autocrine and paracrine pathways. A functional polymorphism of rs4444903A/G can modulate EGF gene expression and associate with multi-

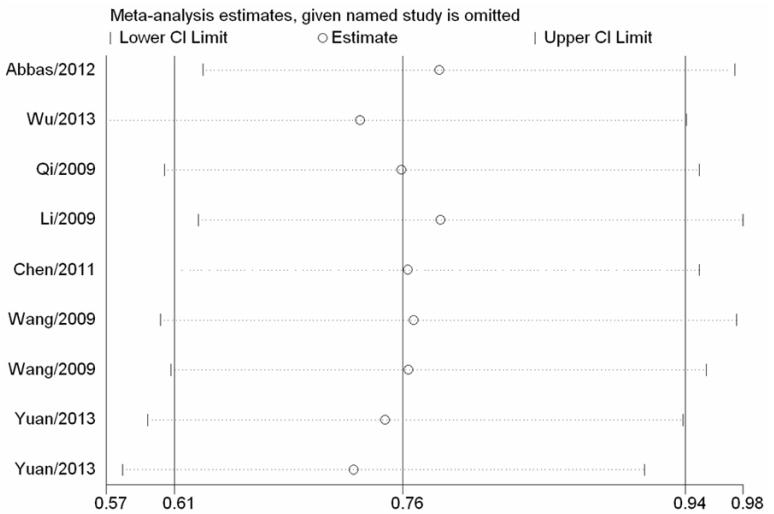

Figure 3. Sensitivity analysis between EGF rs4444903A/G polymorphism and HCC risk.

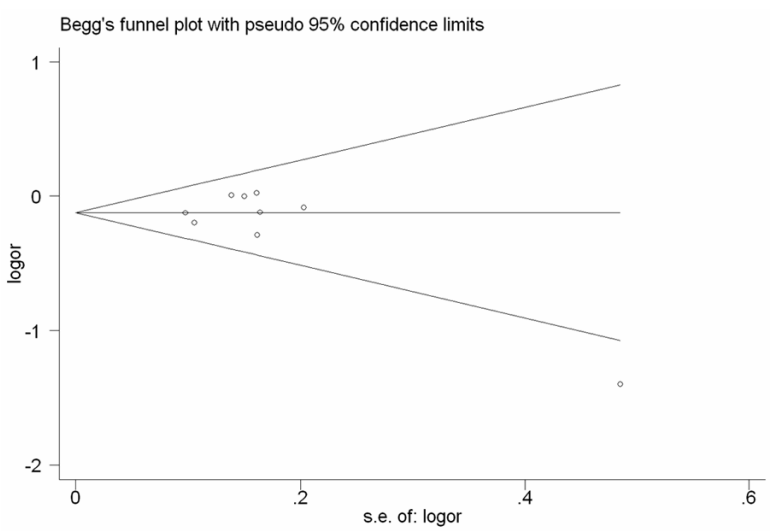

Figure 4. Begg's funnel plot for publication bias test (G-allele vs. A-allele). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size.

ple human malignancies [35-37]. Several studies have attempted to elucidate the mechanism underlying HCC and EGF/EGFR signal pathway. For example, Yoneda et al. [38] reported that the activation of EGF/EGFR signaling pathway via phosphorylation of JNK/SAPK may be closely associated with the histogenesis of CK19-positive HCC. EGF increased the proliferative abilities and invasive properties of HCC cell lines, showing an acquisition of more malignant potential and accounting for the poor prognosis of the HCC patients. Reschke et al. [39] suggested that mitogen-inducible gene-6 (mig-6) is a suppressor of hepatocarcinogenesis, and the loss of mig-6 in primary human liver tumors might be sufficient to generate increased EGFR signaling, which may lead to the initiation and progression of HCC.

Some studies have reported conflicting findings on the association of EGF rs4444903A/G polymorphism with the risk and prognosis of HCC. Tanabe et al. [15] first studied the role of this SNP in a subtype of HCC, suggesting that the number of copies of G was significantly associated with HCC and the severity of cirrhosis (GG+GA vs. AA, hazard ratio = 3.29; 95% CI = 1.29-9.44). Li et al. [30] also found an association between the +61GG genotype and an increased chronic HBV infection-related HCC risk (OR = 2.78, 95% CI = 1.11-6.91). However, in a recent study, Qi et al. [31] demonstrated that there were no significant differences in EGF rs4444903A/G genotype or allelic frequencies or in the tumor stage and invasiveness between HCC patients and healthy controls.

To the best of our knowledge, this study is the first updated analysis that combines all previous related publications to evaluate the relationship between EGF rs4444903A/G polymorphism and HCC risk. In the overall analysis, decreased association was observed between rs4444903A allele and HCC risk in three genetic models. The main finding of our meta-analysis is that the association between EGF rs4444903A/G polymorphism and HCC risk is modified by ethnicity; EGF rs4444903A allele represents a protective factor for HCC in Chinese individuals. We also found an association between EGF rs4444903A/G polymor-

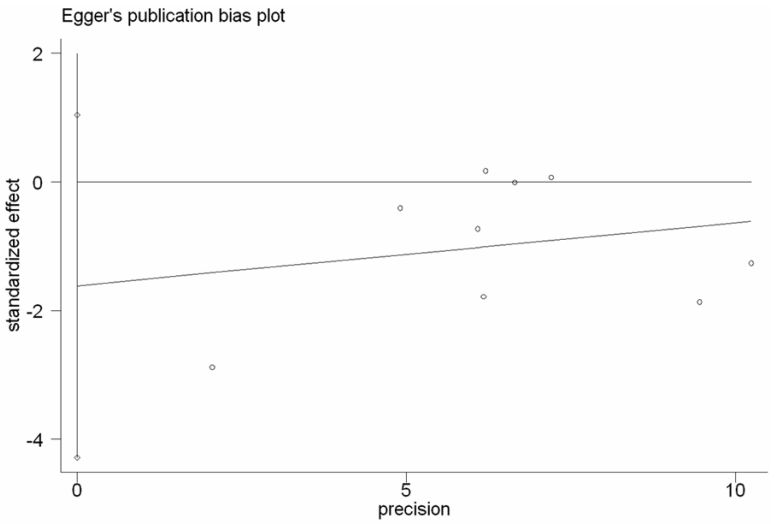

Figure 5. Egger's publication bias plot (G-allele vs. A-allele).

Table 3. Publication bias tests (Begg's test and Egger's test) for EGF rs4444903A/G polymorphism

Compared genotype model	Begg's test		Egger's test	
	z-value	*p*-value	*t*-value	*p*-value
Allelic contrast	1.36	0.175	-2.37	0.050
Homozygote comparison	2.4	0.016	-3.88	0.006
Heterozygote comparison	0.31	0.754	0.04	0.967
Dominant genetic model	1.15	0.251	-1.07	0.320
Recessive genetic model	2.81	0.005	-5.04	0.002

phism and HCC risk in both HB and PB studies.

Meta-analysis has been recognized as an effective method to solve a wide variety of clinical questions by summarizing and reviewing previously published quantitative research; however, our meta-analysis had some limitations. First, the number of published studies included in our meta-analysis was not sufficiently large for a comprehensive analysis, and one study [26] with a small sample size may not have sufficient statistical power to explore the real association. Second, the gene-gene, gene-environment interactions, and even interactions among different polymorphic loci of the same gene may modulate HCC risk. Third, our meta-analysis was based on unadjusted estimates; a more precise analysis should be conducted if individual data are available, which would allow for adjustment by other covariates including age, sex, family history, environmental factors, cancer stage, and lifestyle.

Our meta-analysis also had some advantages. First, a substantial number of patients and controls were pooled from different studies, which significantly increased the statistical power of the analysis. Second, the quality of case-control studies included in the current meta-analysis satisfied our selection criteria. Third, the included control subjects were all healthy individuals, suggesting that the results may be reliable.

In summary, our meta-analysis showed that the EGF rs4444903A allele or/and (AA, AG) genotype was poorly associated with a decreased HCC risk in Chinese individuals. Therefore, further well-designed large studies, particularly referring to gene-gene and gene-environment interactions, are warranted. These future studies should lead to a better and comprehensive understanding of the association between the EGF rs4444903A/G polymorphism and development of risk for HCC.

Disclosure of conflict of interest

None.

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